

A NEW TRICHOMYCETE COMMENSAL WITH A GALATHEID SQUAT LOBSTER FROM DEEP-SEA HYDROTHERMAL VENTS

CINDY LEE VAN DOVER¹ AND ROBERT W. LICHTWARDT²

¹*Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, 02543 and* ²*Department of Botany, The University of Kansas, Lawrence, Kansas, 66045*

ABSTRACT

A new species of eccrinid fungus (Trichomycetes) is described. The fungus is a commensal in the cardiac and pyloric stomachs of the galatheid squat lobster *Munidopsis subsquamosa* (Decapoda, Anomura) collected from deep-sea (2600 m) hydrothermal vents. This is the first record of a trichomycete from depths greater than 50 m. About 40% of the galatheids examined were heavily infested; this proportion varied among the subsamples collected from various vent sites. No fungal thalli were observed in the smallest galatheids.

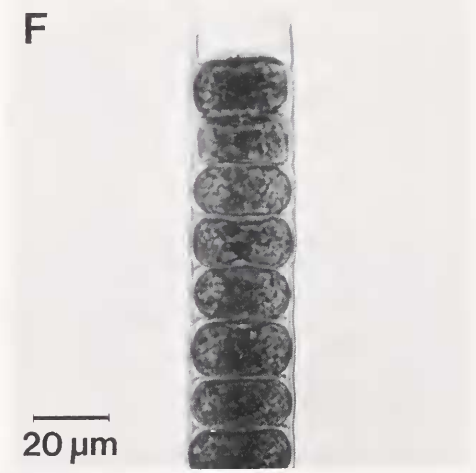
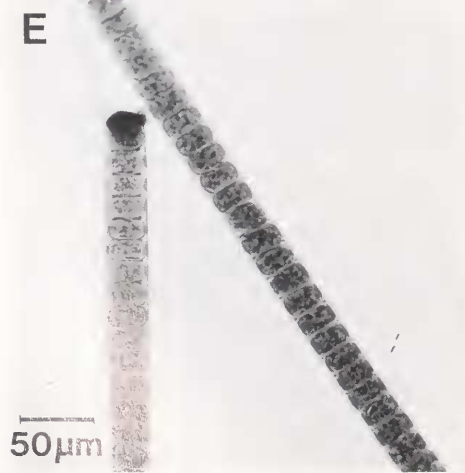
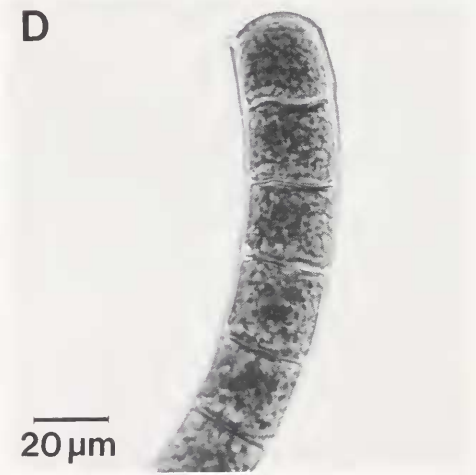
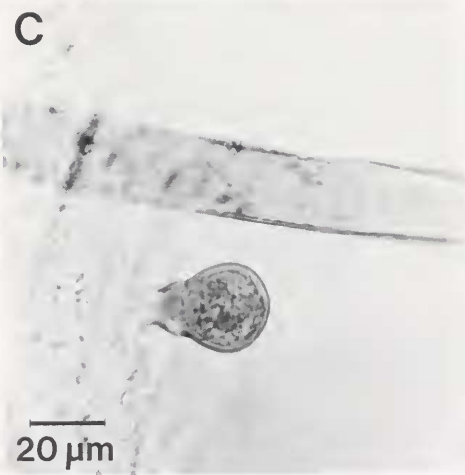
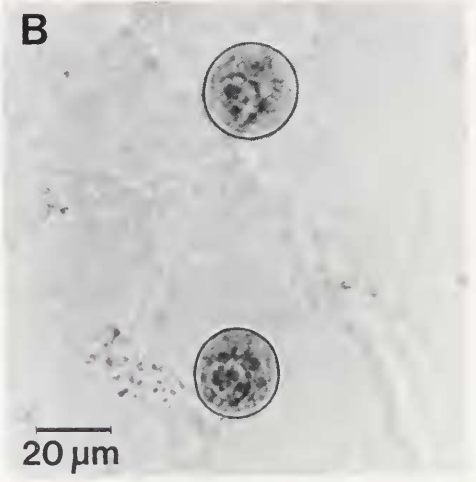
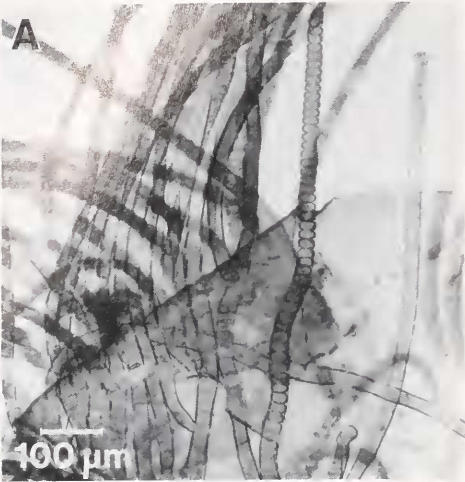
INTRODUCTION

The trichomycetes belong to a class of fungi which is obligately associated with living arthropod hosts. The fungal thalli, attached to the cuticular lining of the foregut and/or hindgut of their host by a holdfast, do not invade the host tissues. Lichtwardt (1976, 1986) summarizes the biology and systematics of the four orders of trichomycetes, including their distribution among the various arthropod groups. Members of the order Eccrinales, characterized by an unbranched, non-septate thallus, are known from diverse arthropods, including insects, millipeds, isopods, amphipods, and anomuran and brachyuran crabs.

The discovery of a new species of eccrinid fungus was the consequence of a routine analysis of gut contents of the galatheid squat lobster *Munidopsis subsquamosa* Henderson. The host galatheids, collected from hydrothermal vent sites in the Pacific Ocean, are thought to belong to a cosmopolitan species complex distributed throughout the world's oceans (J. Ambler, pers. comm.). In addition to regular diet items such as small limpets, crab larvae, polychaetes, and folliculinid protozoans, many of the galatheid stomachs contained long, thin filaments attached to the stomach wall. A superficial resemblance to the filamentous bacterium *Beggiatoa*, known to occur at vent sites and to be grazed by galatheids (Hessler and Smithey, 1983), was noted. Upon further examination, however, the filaments were identified as a new species of eccrinid fungus. This is the first record of a trichomycete from abyssal depths.

MATERIALS AND METHODS

The host galatheids were collected from several different sites during expeditions to Galapagos Rift and East Pacific Rise hydrothermal vents (see Hessler and Smithey, 1983, and Hessler *et al.*, 1985, for descriptions of vent sites). The galatheids forage over the hard, rocky bottom and among the clumps of mussels and clams in the vicinity of the venting water. They were collected inadvertently by the submersible ALVIN as it maneuvered around vent areas, and were subsequently carried to the



surface. Most of the animals were preserved in formalin, then stored in alcohol. The stomachs of four specimens were dissected and preserved in buffered glutaraldehyde. The fungal type specimen was obtained from one of these stomachs. Slides of this stomach were prepared by mounting pieces of material in lactophenol cotton blue, and were examined by light microscopy.

DESCRIPTION

Arundinula abyssicola Van Dover and Lichtwardt, sp. nov.

Thalli ventriculorum Munidopsis subsquamosae (Decapodium Anomurium) ad 2 mm longi, 24–27 μm crassi. Sporangiosporae primae infestationis 12–17 μm longae, 28–38 μm crassae, sub liberatione paene sphaericae factae, 22–27 μm diametro, 4–8-nucleatae, infestationis secundariae 17–24 μm longae, 22–27 μm crassae, item 4–8-nucleatae.

Holotype (glutaraldehyde-preserved stomach) GAL-1, deposited with R. W. Lichtwardt, University of Kansas. Type locality and specimen: Attached to cardiac and pyloric stomach cuticle of *Munidopsis subsquamosa* Henderson; "Rose Garden" hydrothermal vent site at 00°48.0'N, 86°09.9'W (Galapagos Rift), 2600 m.

Unbranched thalli usually not exceeding 2 mm in length, 24–27 μm diameter, with sporulating tips occasionally up to 40 μm diameter. Attached by a centric basal holdfast 10–20 μm long \times 18–22 μm diameter to the cardiac and pyloric stomach of *Munidopsis subsquamosa*. Primary infestation sporangia produced in very long series, their single sporangiospores ellipsoidal but flattened in side view, 12–17 μm long \times 28–38 μm diameter. Mature spores emerge successively through the end wall of the adjacent empty sporangium, becoming almost spherical after release, 22–27 μm diameter and 4–8 nucleate. Secondary infestation sporangiospores 17–24 μm long \times 22–27 μm diameter, 4–8 nucleate. Spore mother-cells persistent and usually becoming fertile.

RESULTS

Some of the stomachs we examined contained possibly one or two thousand thalli, many sporulating prolifically (Fig. 1A). Each sporangium produces a single spore. The primary infestation sporangiospores (Figs. 1B, C) function to disseminate the fungus from one individual anomuran to another after passage from the gut, whereas secondary infestation sporangiospores (Fig. 1D) are capable of germination within the same gut where they are produced, and therefore serve to increase the amount of infestation endogenously.

In the specimens we examined critically, thalli with primary spores were more numerous. After the emergence of a mature (terminal) primary spore, the walls of the vacated sporangia decompose, apparently with the aid of attached bacteria (Fig.

FIGURE 1. A. Thalli in various stages of development attached to a piece of cuticle removed from the host stomach. B. Top view of two primary infestation sporangiospores attached to the stomach lining by means of a rudimentary secreted holdfast that can be seen indistinctly through the spores on their under surfaces. C. Side view of a primary infestation sporangiospore attached to the cuticle by a small holdfast and beginning to germinate. D. Secondary infestation sporangiospores seen developing inside their individual sporangia. E. Thalli that have produced primary infestation sporangiospores. Note the dense bacteria which assist in the decomposition of the sporangial walls after release of spores at the tip of one thallus. F. Thallus tip with primary infestation sporangiospores, the most terminal one beginning to emerge through the end wall of the adjacent empty sporangium.

1E). The production of primary and secondary spores is basipetal, such that the thalli become shorter as the older, terminal spores are released one by one and the empty sporangia degenerate (Fig. 1F).

The tips of thalli producing secondary spores are generally narrower than thalli producing primary spores, and the secondary sporangia can be identified by their square to slightly rectangular appearance in side view. Release of the more or less cylindrical secondary spores occurs most often through a pore or tear in the lateral wall of the sporangium. In some instances we observed rows of several thalli that probably originated from adjacent spores in sporangia lying against the cuticle of the stomach.

The development of thalli from both primary and secondary spores is similar. The spore first attaches to the cuticle by means of a rudimentary secreted holdfast, and germination takes place between this point of attachment and the spore body. As growth of the non-septate, coenocytic thallus continues and more holdfast material is being secreted, the germinated spore, now called a spore mother-cell, persists apically throughout the growth of the thallus (Fig. 2A). After it becomes separated from the main thallus by formation of a cross-wall, the spore mother-cell eventually becomes the terminal sporangium (Fig. 2B). In many other eccrinid genera the spore mother-cell disintegrates before the thallus reaches maturity.

It is possible in *A. abyssicola* to distinguish those thalli that originated from primary spores from those produced by secondary spores, so long as the spore mother-cell is still present. Primary spore mother-cells are positioned at the tip of the thallus in line with the axis, and are rounded to sometimes flattened at the apical end (Figs. 2A, B), whereas secondary spore mother-cells tend to be more angular and have a reflexed position due to the area on the spore wall where attachment and germination took place (Fig. 2C).

We did not find thalli of *A. abyssicola* attached to the hindgut lining of *M. sub-squamosa*, a condition reported to occur in other species of *Arundinula* except for *A. galathea* (also commensal in a galatheid squat lobster). Nor did we find the oval to ellipsoidal sporangiospores with single appendages at each pole that have been seen, though rarely, in the exuviae of the respective anomuran hosts of *A. capitata* and *A. washingtoniensis*.

Arundinula abyssicola is distinct from the other five valid species of the genus on the basis of the shape and dimensions of its spores and its unusual anomuran host. It also can be distinguished from most other species of *Arundinula* by the morphology of its thalli and the shape and location of its holdfast (Fig. 2D). Morphological characteristics of *Arundinula* species are summarized in Table I.

DISCUSSION

The genus *Arundinula* includes five species in addition to *A. abyssicola* described here. They are known to occur in several families of shallow-water marine anomurans as well as in freshwater crayfish (reviewed in Lichtwardt, 1986). The basic morphology of the new eccrinid species is clearly allied with that of the other members of the genus, despite the extraordinary depths at which it was collected and the unusual physico-chemical environment of the hydrothermal vents.

Not all of the galatheids collected from the Galapagos Rift and the East Pacific Rise contained fungi (Table II). About 40% of the animals were infested. This proportion was the same in both male and female subpopulations (Table III). A greater proportion of gravid females were infested than non-gravid females.

No fungi were found in the smallest individuals (Table III). It may be that these

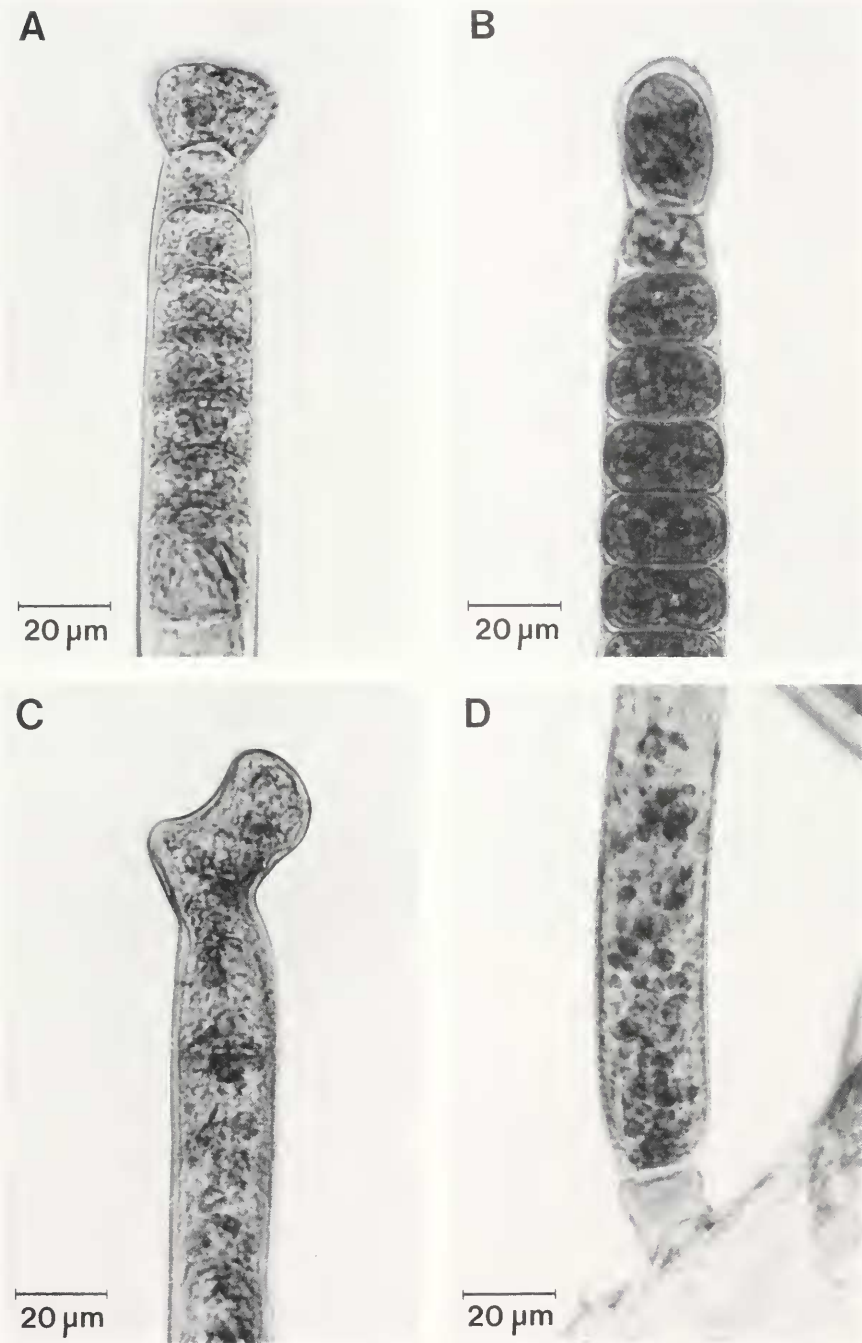


FIGURE 2. A. Tip of a thallus that developed from a primary infestation sporangiospore, beginning to form septa so as to produce sporangia. At this stage each resulting cell is uninucleate. B. A primary spore mother-cell that has become a sporangium and contains a single spore. C. Developing thallus tip with a reflexed secondary spore mother-cell. D. Holdfast anchoring a mature thallus to the stomach lining.

TABLE I

Morphological characteristics of Arundinula species summarized from Lichtwardt (1986) and this study

Species	Host	Primary infestation sporangiospores		Secondary infestation sporangiospores			Thalli	
		Shape	Size (μm)	Shape	Size (μm)	Form	Length (mm)	Diameter (μm)
<i>A. capitata</i> Léger & Duboscq, 1906 <i>A. galathea</i> Manier & Ormières ex Manier, 1968 <i>A. haplogaster</i> Hibbits, 1978	Paguridae (<i>Eupagurus</i> , <i>Pagurus</i>)	flat, disk-like	—	elongate	13–15(–30) (length)	Foregut: thick-walled Hindgut: ≥10	1–2(–3) ≥10	17–22, 23–30 9–17, 35–60
	Galatheidæ (<i>Galathea</i>)	round or oval	27–31	oval	45–65 × 30–45	Rigid, thick-walled; tapered	≤3	27–47 base, 50–83 apex
	Lithodidae (<i>Haplogaster</i>)	oval	20–35(–43) × 5–7.5	doliiform	20–35 × 17–35	Foregut and anterior part of hindgut: a) curved b) straight Hindgut: a) undulate b) coarse, tapered c) tapered d) thick-walled	3 — 6 15 7 8	16–35 — 12–25 41–50 base 17–25 apex 37–43 base, 16–21 apex 55–105 base, 9 apex 20
	Astaciidae (<i>Orconectis</i>)	spherical	24–34	swollen proximally doliiform	36 × 20	Thin-walled	up to 20	
<i>A. orconectis</i> Lichtwardt, 1962 <i>A. washingtoniensis</i> Hibbits, 1978	Paguridae (<i>Paguristes</i>)	ellipsoidal with long (120 μm), tapering appendage at each pole	—		various: 20–35 × 10–20 40–80 × 14–19 135–390 × 11–20	Foregut: a) thick-walled b) thick-walled c) very-thick-walled Hindgut: a) thick-walled b) thick-walled Thin-walled	1.8 4.2 5–6 6.5 13.5 <2	22–45 17–27 60–120 20–45 11–30 24–27, sporulating tips up to 40
<i>A. abyssicola</i> sp. nov.	Galatheidæ (<i>Munidopsis</i>)	spherical	22–27	cylindrical	17–24 × 22–27			

TABLE II

Eccrinid infestations of galatheid populations at hydrothermal vent sites in the eastern Pacific Ocean

	Year	# Infestations/ # <i>M. subsquamosa</i> examined	% Infested
Galapagos Rift (00°48.0'N; 86°09.0'W)			
Garden of Eden	1979	1/3	33%
Mussel Bed	1979	0/1	0%
Rose Garden	1979	0/1	0%
Rose Garden	1985	4/5	80%
East Pacific Rise (20°50.0'N; 109°06.0'W)			
Clam Acres	1982	8/23	35%
Clam Acres	1985	1/4	25%
TOTAL		14/37	38%

individuals are molting so frequently that the fungus is shed with the cuticular lining of the stomach more rapidly than it can be re-acquired, or that the animals simply have not had a chance to be exposed to the fungus at all.

The Rose Garden site in 1985 seems to have a higher proportion of infestations relative to other sites (Table II). Greater densities of galatheids occurred at Rose Garden in 1985 than in 1979 (R. Hessler, pers. comm.); large host populations may facilitate fungal infestations. The more sparse populations of non-vent deep-sea galatheids may be expected to have a very low incidence of infestation. The age of a vent community and its galatheid population may also influence the incidence of infestation. At older vents, more time will have been available for transmission of the fungus throughout the local population.

The nature of the relationship between eccrinids and their host is not clear. The early literature referred to the fungi as parasites, but neither detrimental nor beneficial effects by eccrinids have been demonstrated. The fungus and host are not symbiotic in the sense of the symbiosis between other vent organisms such as the clams, mussels,

TABLE III

Eccrinid infestations of M. subsquamosa by host sex and size

	# Infestations/ # <i>M. subsquamosa</i> examined	% Infested
Sex		
Males	4/10	40%
Females		
non-gravid	3/13	23%
gravid	6/10	60%
combined	9/23	39%
Size (rostral-carpace length)		
> 50 mm	3/7	43%
40–50 mm	6/12	50%
30–40 mm	3/8	37%
<30 mm	0/4	0%

or vestimentiferans and chemoautotrophic bacteria. These vent animals are thought to derive much or all of their nutrition from their symbionts (reviewed in Cavanaugh, 1985), while the galatheid is known to be an active predator/scavenger. But it may be that subtle beneficial effects are present, such as the synthesis of complex nutrients by the fungus which might be used by their hosts when external nutrients are sub-optimal.

ACKNOWLEDGMENTS

We thank Professor Donald P. Rogers for preparation of the Latin diagnosis. This is contribution number 6184 of the Woods Hole Oceanographic Institution.

LITERATURE CITED

- CAVANAUGH, C. M. 1985. Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. Pp. 373-388 in *Hydrothermal Vents of the Eastern Pacific: An Overview*, M. L. Jones, ed. *Biol. Soc. Wash. Bull.* (6).
- HESSLER, R. R., AND W. M. SMITHEY, JR. 1983. The distribution and community structure of megafauna at the Galapagos Rift hydrothermal vents. Pp. 735-770 in *Hydrothermal Processes at Seafloor Spreading Centers*, P. A. Rona, K. Bostrom, L. Laubier, and K. L. Smith, eds. Plenum Press, New York.
- HESSLER, R. R., W. M. SMITHEY, JR., AND C. H. KELLER. 1985. Spatial and temporal variation of giant clams, tube worms and mussels at deep-sea hydrothermal vents. Pp. 411-428 in *Hydrothermal vents of the Eastern Pacific: An Overview*, M. L. Jones, ed. *Biol. Soc. Wash. Bull.* (6).
- LICHTWARDT, R. W. 1976. Trichomycetes. Pp. 651-671 in *Recent Advances in Aquatic Mycology*, E. B. Gareth Jones, ed. Elek Science, London.
- LICHTWARDT, R. W. 1986. *The Trichomycetes, Fungal Associates of Arthropods*. Springer-Verlag, New York. 343 pp.